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Patient and disease precursors and clinical predictors of prolonged cytopenias in patients with aggressive B-cell non-Hodgkin's lymphoma treated with chimeric antigen receptor T-cell therapy

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PATIENT AND DISEASE PRECURSORS AND CLINICAL PREDICTORS OF PROLONGED CYTOPENIAS IN PATIENTS WITH AGGRESSIVE B-CELL NON-HODGKIN'S LYMPHOMA TREATED WITH CHIMERIC ANTIGEN RECEPTOR T-CELL THERAPY

by

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DEDICATION

I dedicate this thesis to my parents, Regis and Regina Saucier. I would not be where I am today without their unwavering support, generosity, and love. They have believed in me every step of the way, even when I struggled to believe in myself. Mom and Dad, no words can express how grateful I am to you both, but I will start with thank you.

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ABSTRACT

Introduction: Chimeric antigen receptor (CAR) T-cell therapy is a new treatment for hematologic malignancies including aggressive B-cell non-Hodgkin's lymphoma (NHL). Although it has provided an effective treatment option for patients who have few options, CAR T-cell therapy does have many associated toxicities. Prolonged cytopenias are one of the lesser understood toxicities that can affect upwards of 40% of patients. **Methods:** In this retrospective study, we reviewed 106 patients who received commercial CAR T-cell therapy between November 2017 and September 2019. Prolonged cytopenias were defined as having absolute neutrophil count (ANC) <1000/mm^3, platelets (PLT) <50,000/mm^3, and/or hemoglobin (Hgb) <10 g/dL at least once after 30 days post-CAR T-cell infusion. Furthermore, if only one incidence of cytopenia was recorded 30 days post infusion, we required that the patient had to have received either a transfusion or granulocyte-colony stimulating factor (GCSF) after the date of the recorded cytopenic value to be considered a part of the cytopenic cohort. **Results:** 22 patients met the criteria of having prolonged cytopenias. 64% of the cytopenic cohort had >1 type of prolonged cytopenias. Anemia was the most prevalent affecting 72% of cytopenic patients. The length of time from diagnosis of aggressive B-cell NHL to date of CAR T-cell infusion

was found to be positively correlated with an increased risk of developing prolonged cytopenias following CAR T-cell therapy. Additional risk factors associated with an increased risk of delayed cytopenias by univariate analysis included neutropenia on the day of infusion (day 0), a high C-reactive protein (CRP) before lymphodepletion and on day 0, day 0 PLT count, and Hgb before lymphodepletion and on day 0. On multivariate analysis, only high CRP before lymphodepletion was associated with an increased risk of prolonged cytopenias while high ferritin and PLT values on day 0 were associated with not developing prolonged cytopenias. There was no statistical difference between the cytopenic and non-cytopenic cohorts in rates of progression free survival (PFS) and overall survival (OS). Also, no difference was seen in rates or severity of other toxicities between cohorts. 41% of the cytopenic cohort experienced infectious complications post-infusion with one patient dying from their infectious complications. However, there was no association with incidence of infection and prolonged cytopenias when compared to the incidence of infection in the non-cytopenic cohort. **Conclusions:** A longer time from diagnosis of aggressive B-cell NHL to time of CAR T-cell infusion was associated with prolonged cytopenias while number of lines of prior chemotherapy and rate of prior high dose chemotherapy with an autologous stem cell transplant (HD-ASCT) were not associated. It would be valuable to confirm this association and why it is associated since the other two factors were not. We lacked bone marrow biopsies before CAR T-cell infusion and did not have bone marrow biopsies for many patients after CAR T-cell infusion. It would be beneficial to collect data regarding bone marrow biopsies from these time points to highlight any changes that could be related to CAR T-cell therapy.

Cytogenetic information of individual patient's diseases would be worth analyzing to help determine if there are biological factors associated with prolonged cytopenias in response to CAR T-cell therapy. Additional studies should investigate the laboratory values we found to have associations with either cohort to help identify possible predictive values providers could use to identify patients at higher risk of having prolonged cytopenias. There is also a need to see if specific prior chemotherapy regimens increase a patient's risk of having prolonged cytopenias. Overall, since prolonged cytopenias after CAR T-cell infusions have not been heavily investigated, further investigation is needed to better understand the predictive factors and identify possible mechanisms of prolonged cytopenias seen in CAR T-cell patients.

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LIST OF ABBREVIATIONS

AE ..... Adverse Event

ALC ..... Absolute Lymphocyte Count

ALL ..... Acute Lymphoblastic Leukemia

ANC ..... Absolute Neutrophil Count

BEAM ..... Carmustine, Etoposide, Cytarabine, Melphalan

BH ..... Benjamini-Hochberg (Procedure)

BR ..... Bendamustine, Rituximab

CAR ..... Chimeric Antigen Receptor

CHL ..... Classical Hodgkin's Lymphoma

CR ..... Complete Response

CRP ..... C-reactive Protein

CRS ..... Cytokine Release Syndrome

CT ..... Computed Tomography

CTCAE ..... Common Terminology Criteria for Adverse Events

DFCI ..... Dana-Farber Cancer Institute

DLBCL ..... Diffuse Large B-cell Lymphoma

D5PS ..... Deauville Five Point Scale

ECOG ..... Eastern Cooperative Oncology Group

EFS ..... Event-Free Survival

FDA ..... Food and Drug Administration

FDG ..... Fluorodeoxyglucose

G-CSF .....Granulocyte-Colony Stimulating Factor

HD-ASCT ..... High Dose Chemotherapy with an Autologous Stem Cell Transplant

Hgb.....Hemoglobin

HGBL.....High Grade B-cell Lymphoma

HLA .....Human Leukocyte Antigen

ICANS .....IEC-Associated Neurotoxicity Syndrome

IEC ..... Immune Effector Cell

IL .....Interleukin

IPI.....International Prognostic Index

IRB ..... Institutional Review Board

LDH .....Lactate Dehydrogenase

LLN.....Lower Limits of Normal

M-BACOP .....Methotrexate, Bleomycin, Doxorubicin, Cyclophosphamide, Prednisone

MDS .....Myelodysplastic Syndrome

MHC .....Major Histocompatibility Complex

MRSA .....Methicillin-resistant Staphylococcus aureus

NHL .....Non-Hodgkin's Lymphoma

O-CHOP..... Obinutuzumab, Cyclophosphamide, Doxorubicin, Vincristine, Prednisone

OS .....Overall Survival

PD ..... Progressive Disease

PET .....Positron Emission Tomography

PFS .....Progression Free Survival

PLT .....Platelets

PMBCL .....Primary Mediastinal B-cell Lymphoma

PR.....Partial Response

RBC .....Red Blood Cell

R-CHOP.....Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, Prednisone

R-CVP..... Rituximab, Cyclophosphamide, Vincristine, Prednisone

R-cytarabine .....Rituximab, Cytarabine

R-DHAC .....Rituximab, Dexamethasone, Cytarabine, Carboplatin

R-DHAP.....Rituximab, Dexamethasone, Cytarabine, Cisplatin

R-EPOCH…… Rituximab, Etoposide, Prednisone, Vincristine, Cyclophosphamide, Doxorubicin

R-ESHAP.....Rituximab, Etoposide, Solu-medrone, Cytarabine, Cisplatin

RICE .....Rituximab, Isofamide, Carboplatin, Etoposide

R-GDP ..... Rituximab, Gemcitabine, Dexamethasone, Cisplatin

R-gem/navelbine .....Rituximab, Gemcitabine, Navelbine

R-GemOx ..... Rituximab, Gemcitabine, Oxaliplatin

R-DHAP.....Rituximab, Dexamethasone, Cytarabine, Cisplatin

R-VIC..... Rituximab, Etoposide, Ifosfamide, Carboplatin

SD .....Stable Disease

TFL .....Transformed Follicular Lymphoma

THRLBCL ..... T-cell/Histiocyte Rich Large B-cell Lymphoma

URI..... Upper Respiratory Infection

UTI..... Urinary Tract Infection

INTRODUCTION

Aggressive B-cell Non-Hodgkin's Lymphoma

Non-Hodgkin's Lymphoma (NHL) accounts for approximately 4% of all cancer, and an expected 77,240 people will be diagnosed with NHL this year.[1,2] Moreover, NHL is the eighth and ninth most common cause of cancer-related death in women and men, respectively.[2] NHL is a broad classification of lymphomas that encompasses more than 90 specific types of lymphoma.[3] NHL are grouped into two categories: indolent and aggressive lymphomas.[4] Indolent lymphomas are distinguished by having long survival time, often many years, and quick responses to treatments, but a lack of a curative standard therapies.[4] Conversely, aggressive lymphomas typically exhibit rapid progression without therapy and can be cured with standard chemotherapies.[4] However, survival is short when patients are not able to be cured by chemotherapy.[4]

More specifically, B-cell lymphomas make up approximately 85% of all NHL in the United States.[5] The category of aggressive B-cell lymphoma includes the diagnoses of diffuse large B-cell lymphoma (DLBCL), transformed follicular (TFL) or transformed marginal zone lymphoma, high-grade B-cell lymphoma (HGBL), primary mediastinal B-cell lymphoma (PMBCL), T-cell/histiocyte-rich large B-cell lymphoma (THRLBCL), and several others.[6,7] DLBCL is the most common of these subtypes and constitutes about a third of all NHL.[2]

DLBCL is classified as diffuse proliferation of large cells with high mitotic rates.[2] The tumor cells' large size is the only feature tying DLBCL together; the different types

of DLBCL may otherwise have varying morphologies.[2] DLBCL can either occur due to transformation from indolent lymphomas or can be de novo in origin. [8] Transformation can also be seen from other indolent lymphomas including marginal zone lymphoma.[7] Studies have found that patients with de novo DLBCL have a better prognosis than transformed patients.[8] Conversely, HGBL is associated with worse outcomes than DLBCL even after receiving intensive chemotherapy regimens.[9,10] Approximately 30% of patients diagnosed with HGBL previously had another form of B-cell NHL.[10]

PMBCL is another form of aggressive B-cell NHL most commonly found in the mediastinum of young women.[11] It often appears as groups of large malignant cells with abundant cytoplasm that is typically separated by eosinophilic fibrosis.[11] Patients with PMBCL typically have better survival rates than patients with DLBCL.[11] Another form of aggressive B-cell NHL, called gray zone lymphoma, gets its name from having genetics similar to both PMLBCL and classical Hodgkin's Lymphoma (CHL).[11] It is typically seen in young men and has a worse prognosis than that of PMLBCL and CHL.[11]

Lastly, THRLBCL is another form of aggressive B-cell NHL that is most commonly seen in middle aged men.[11] THRLBCL often has involvement in the liver, spleen, and bone marrow.[12] Additionally, THRLBCL and another lymphoma, nodular lymphocyte predominant Hodgkin lymphoma, appear to be biologically connected with THRLBCL being the more aggressive of the two and having a poorer prognosis.[11]

Standard Treatment for Aggressive B-cell NHL Prior to CAR T-cell Therapy

Regarding treatment for aggressive B-cell NHL, almost all patients are treated with an anti-CD20 monoclonal antibody and induction regimen containing anthracycline.[13] The widely accepted first line therapy is R-CHOP, which stands for the following pharmaceutical agents: rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone.[8,13] For patients with aggressive B-cell NHL, approximately 50-60% achieve and maintain a durable complete response (CR).[6,8,14] Patients with relapse or refractory aggressive B-cell NHL after their first line of therapy not only have a low cure rate with typical therapy options but, without further treatment, also have a life expectancy in the range of months.[8,13,14]

The second treatment option for patients with relapsed or refractory aggressive B-cell NHL would be undergoing a salvage chemoimmunotherapy regimen with the goal of proceeding to high dose chemotherapy with an autologous stem cell transplant (HD-ASCT).[8] Some common second line treatment regimens are rituximab, isofamide, carboplatin, and etoposide (RICE); rituximab, dexamethasone, cytarabine, and cisplatin (R-DHAP); and rituximab, gemcitabine, dexamethasone, and cisplatin (R-GDP).[13] All three have similar efficacy with about one-third of patients continuing to HD-ASCT.[13]

In regards to the clinical importance of HD-ASCT, one retrospective study found that the overall survival (OS) and the progression free survival (PFS) in patients with primary relapsed DLBCL who received salvage chemotherapy without proceeding to HD-ASCT was 38% and 29%, respectively.[15] Meanwhile, patients who received both salvage chemotherapy and HD-ASCT had an OS of 65% and a PFS of 60%.[16] However,

another study conducted by Gisselbrecht and colleagues found that patients with relapsed large B-cell lymphoma who relapsed within the first year of being diagnosed had a 3-year event-free survival (EFS) of 20% while the patients who relapsed more than a year after being diagnosed had a 3 year EFS of 45%.[15] The researchers also found that patients with relapsed large B-cell lymphoma who had received prior rituximab had a 3-year EFS of 20% compared to the 3-year EFS of 45% seen in their counterparts that had not received a rituximab-containing regimen.[15] These findings supported the need for developing more treatment options for patients with relapsed or refractory aggressive B-cell NHL.

Chimeric Antigen Receptor T-cell History

Chimeric antigen receptor (CAR) T-cell therapy is a new treatment with relatively fast development since the discovery of T-cells in 1961. T-cell engineering began in 1992 when immunologist Michel Sadelain began using retroviral vectors along with other genetic engineering tools to enhance the effectiveness of T-cells in the body.[17] In 1993, the first generation of CARs were developed; they were not clinically effective since they were unable to survive in vivo.[17] However, in 1998, a costimulatory molecule was added to engineered T-cells, which allowed them to stay active in the human body[17] The first effective CAR T-cells were developed in 2002 for prostate cancer.[17]

Shortly thereafter, in 2003, the second generation of CARs were built to target CD19 cells and were proven to kill leukemia cells in mice.[17] The first human study using CARs was published in 2013 and in 2014, the Food and Drug Administration, FDA, gave CD-19 directed CAR T-cells Breakthrough Designation[17] In 2017, CAR T-cell therapy

was approved first for children and young adults with relapsed or refractory acute lymphoblastic leukemia, ALL, and then for patients with relapsed or refractory DLBCL who had previous had two lines of systemic therapy.[17,18]

CAR T-cell Structure and Function

In regard to their structure, CARs most commonly have an extracellular target-binding domain, a hinge region, a transmembrane domain, and one or more intracellular domains.[19] The extracellular target-binding domain, typically a single-chain variable fragment, dictates the capability of the CAR to bind to an antigen, and when bound, activates the T cells.[19,20] This region allows for the T-cell to recognize its target antigens without using the major histocompatibility complex (MHC) or the human leukocyte antigen (HLA) both of which can be downregulated by tumors as a method of evading the immune system.[20,21]

The hinge region is also extracellular and serves to separate the binding domain from the transmembrane domain.[19] The hinges are typically immunoglobulin-like and also provide stability to allow for efficient CAR expression and function.[19] The transmembrane domain may be the least characterized aspect of the CAR with its primary role being to secure the CAR in the T cell membrane.[19] However, there is some evidence suggesting that it may also have a pertinent role in the CAR T-cell function.[19]

The intracellular portion contains both a T-cell receptor signaling domain and a co-stimulatory domain.[21] The costimulatory domain helps to regulate the survival and effector function of the T-cells.[19] The costimulatory domains have distinct properties that

make it improbable that some costimulatory domain will serve all desired purposes.[19] Therefore, substantial research and understanding should be used when determining the best intracellular portion not only per disease, but also potentially per individual.

The function of the CAR when introduced to a T-cell is that the T-cell has greater antigen specificity and contains the signals necessary for full T-cell activation.[19] Since the creation of CARs, there are now three distinct generations. These generations are distinguished by the number of costimulatory domains: the first generation contains CD3z only, the second generation contains one costimulatory domain and CD3z, and the third generation contains greater than one costimulatory domain along with CD3z.[19] Both FDA approved CAR T-cell therapies to treat relapsed or refractory DLBCL, Yescarta, generic name axicabtagene ciloleucel, and Kymriah, generic name tisagenlecleucel, are second generation CARs.[22–24]

Clinical Course of CAR T-cell Therapy

The first step when considering CAR T-cell for a patient is determining if they are eligible to receive the therapy.[25] This is determined based on a variety of tests and screenings and may vary slightly at different treatment centers.[25] At Dana-Farber Cancer Institute (DFCI), where the research performed in this thesis was conducted, some of the eligibility criteria includes: having a confirmed diagnosis of DLBC, PMLBCL, HGBCL, or TFL, having failed at least two lines of chemotherapy or relapsed within twelve months of receiving an HD-ASCT, and lastly, having sufficient cardiac, pulmonary, and organ function.[26]

After a patient has been deemed eligible, the next step is collecting the T-cells. The T-cells are removed from the patient's blood using a processed called apheresis where their blood is taken from the patient's body, the targeted components, in this case T-cells, are removed, and the remaining blood is returned to the patient.[25] Once collected, the T-cells are transported to a laboratory where they are genetically engineered to express CARs.[25] Next, the CAR T-cells undergo multiplication in the laboratory until there are millions, which may take a few weeks, and then they are frozen and sent to the hospital where infusion takes place.[25]

Prior to infusion, patients undergo a lymphodepletion regimen consisting of varying chemotherapies that remove immunosuppressive elements and create room within the patient's immune system to allow for expansion and proliferation of the CAR T-cells.[21,25] For both axicabtagene ciloleucel and tisagenlecleucel, the chemotherapy agents fludarabine and cyclophosphamide are used before infusion of the CAR T-cells.[18,27] The reasoning behind lymphodepletion before infusion is that it might increase the efficacy of the CAR T-cells by hampering the start of a T cell immune response against the murine single-chain variable fragment aspect of the CAR.[21] After completing lymphodepletion, the patient is ready to undergo infusion of the CAR T-cells.

The process of the CAR T-cell infusion is similar to a blood transfusion, however, patients are admitted for anywhere from a few days to several weeks in order to monitor and treat any side effects or complications that may occur as a result of the infusion.[25] These side effects will be discussed at length in the next section. Once the appropriate time has passed without any sign of complications, or complications have been resolved,

the patient is discharged and must remain close to the hospital for at least thirty days post-infusion due to the common need for readmission because of additional side effects.[25]

A clear protocol for long-term follow-up for patients who have received CAR T-cell therapy has not been established due to the newness of the therapy and the variables of an individual patient's response.[21] It is recommended by the leading experts in CAR T-cell therapy to use positron emission tomography (PET)/computed tomography (CT) to determine a patient's response to treatment and, if further treatment is needed, decrease time to the next intervention.[21] The intervals at which imaging occurs depends on treatment responses, but has been recommended for all patients at one month and three months post-infusion.[21] For axicabtagene ciloleucel and tisagenlecleucel, disease progression or relapse is mostly seen within the first three to six months post-infusion.[21,28,29] Until further long-term data is collected, it is suggested that imaging for patients in CR after three months be done when deemed appropriate by the physician, whereas PET/CT imaging every three months is recommended for patients not in CR.[21]

CAR T-cell Therapy Toxicities

CAR T-cell therapy is of great clinical value due to its ability to provide significant clinical responses, particularly in patients who may have previously been without effective later lines of therapy.[30] However, it comes with a set of distinct toxicities that can range from mild to severe to sometimes fatal.[30] These toxicities tend to be distinct from the toxicities seen in more traditional treatment options, such as

chemotherapy or monoclonal antibodies.[30] One main mechanism that may be responsible for some of the toxicities is an on-target, off-tumor effect where the tumor associated antigen is also expressed on normal tissue that can then lead to tissue damage.[30,31] Additionally, CAR T-cells may unpredictably cross-react with proteins that are not found on the tumor cells and cause damage to non-cancerous tissue.[31] Table 1 contains a comprehensive list of CAR T-cell toxicities.

Table 1. CAR T-cell Toxicities

Organ system	Toxicities
Constitutional	• Fever • Fatigue and malaise • Headache
Cardiovascular	• Sinus tachycardia • Hypotension • Decreased left ventricular ejection fraction • Arrhythmias • QT prolongation • Troponinemia
Respiratory	• Hypoxia • Dyspnea • Increased respiratory rate • Respiratory failure • Pleural effusions • Capillary leak syndrome
Renal	• Increased serum creatinine • Renal insufficiency • Hyponatremia • Hypokalemia • Hypophosphatemia • Tumor lysis syndrome
Hepatic and Gastrointestinal	• Increases in liver transaminases: elevated aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, or direct bilirubin • Nausea, vomiting • Diarrhea
Hematologic	• Anemia • Thrombocytopenia • Neutropenia • B-cell aplasia • Hypogammaglobulinemia • Prolongation of partial thromboplastin time (PTT) or prothrombin time (PT) • Decreased fibrinogen • Disseminated intravascular coagulation (DIC) • Hemophagocytic lymphohistiocytosis
Immunologic	• Risk of viral infections • Risk of bacterial infections • Risk of fungal infections
Musculoskeletal	• Creatine phosphokinase (CPK) elevation • Myalgias
Neurologic	• Delirium, encephalopathy • Somnolence, obtundation • Cognitive disturbance • Dysphasias • Tremors • Ataxia • Myoclonus • Focal motor and sensory defects • Seizures • Cerebral edema

List of possible toxicities a patient may experience after receiving CAR T-cell therapy. Taken from Brudno JN and Kochenderfer JN, 2019

The two most commonly occurring toxicities are cytokine release syndrome (CRS) and immune effector cell (IEC)-associated neurotoxicity syndrome (ICANS).[30,32,33] CRS is a systemic inflammatory response that is noted to cause high fevers, hypotension, hypoxia, and cardiac and other organ dysfunctions.[32] CRS is believed to be caused by the release of cytokines from not only the infused CAR T-cells, but also from other immune cells, such as macrophages, that may produce cytokines in response to the cytokines produced by the CAR T-cells.[30–32] In ZUMA-1, the phase 2 clinical trial that led to the FDA approval of axicabtagene ciloleucel, 94% of patients had CRS.[29] The rate of patients experiencing grade 3 or above CRS was 11% of axicabtagene ciloleucel patients in the ZUMA-1 trial and 22% of tisagenlecleucel patients in the JULIET trial, the phase 2 clinical trial that also lead to FDA approval.[28,29]

Increased circulating interleukin (IL)-6 and interferon γ.[34] Tocilizumab, an anti-IL-6 humanized monoclonal antibody, is used with or without corticosteroids to reverse CRS.[34] Tocilizumab is considered the first line treatment for CRS due to its more rapid response.[34] Corticosteroids are considered second line although they are an effective treatment option for CRS.[34] This is because corticosteroids can suppress T-cell function and induce apoptosis.[30]

ICANS is characterized as a toxic encephalopathic state that leads to symptoms of confusion, delirium, seizures ,and cerebral edema.[30] However, ICANS has diverse presentations and does not target any one specific area in the central nervous system.[31,32] Other commonly seen effects of ICANS include but are not limited to headaches, confusion, somnolence, hallucinations, dysphasia, ataxia, apraxia, tremors, and seizures.[32]

Publications note the rate of ICANS as being anywhere from 0% to 50%. [31] The rate of grade 3 and 4 ICANS for axicabtagene ciloleucel patients in the ZUMA-1 study is 32% and 12% for tisagenlecleucel patients in the JULIET trial.[28,29] Corticosteroids are used in the treatment of ICANS.[30]

Prolonged Cytopenias in CAR T-cell Therapy Patients

Another common group of toxicity seen in CAR T-cell patients is hematopoietic toxicities including neutropenia, anemia, and thrombocytopenia.[35] More commonly referred to as cytopenias, some studies suggest that upwards of 90% of patients experience cytopenia after CAR T-cell infusion.[21] The United States Department of Health and Human Services publishes a Common Terminology Criteria for Adverse Events (CTCAE) that helps standardize adverse event (AE) reporting.[36] Each AE is graded from one to five with one being mild and five meaning death occurred relating to the AE.[36] Table 2 provides the definition of the cytopenias along with the CTCAE criteria for grading each cytopenia.[36]

Table 2. Cytopenia Definitions and Grading based on CTCAE Criteria

CTCAE Term	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Anemia	Hemoglobin (Hgb) <LLN – 10.0 g/dL	Hgb<10.0-8.0 g/dL	Hgb <8.0 g/dL	Life-threatening consequences; urgent intervention indicated	Death
Definition: A disorder characterized by a reduction in the amount of hemoglobin in 100 ml of blood. Signs and symptoms of anemia may include pallor of the skin and mucous membranes, shortness of breath, palpitations of the heart soft systolic murmurs, lethargy, and fatigability.					
Neutropenia	Absolute Neutrophil Count (ANC) <LLN – 1500/mm^3	ANC <1500 – 1000/mm^3	ANC <1000-500/mm^3	ANC <500/mm^3	N/A
Definition: A finding based on laboratory test results that indicate a decrease in number of neutrophils in a blood specimen					
Thrombocytopenia	Platelets (PLT) <LLN – 75,000/mm^3	PLT <75,000 – 50,000/mm^3	PLT <50,000 – 25,000/mm^3	PLT <25,000/mm^3	N/A
Definition: A finding based on laboratory test results that indicate a decrease in number of platelets in a blood specimen					

LLN: Lower Limit of Normal

Adapted from Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0, 2017

Cytopenias typically occur within the first 28 days after infusion, but may take three months or more to improve.[21] Identifying the cause of these cytopenias is a challenge because they could be an effect of heavy pretreatment, the cytotoxic lymphodepletion regimens, an overall effect of CAR-Ts, or a combination of the three.[21] With the understanding that the chemotherapy used during lymphodepletion is known to cause cytopenias, it is important to note that patients who did not receive lymphodepleting chemotherapy have also had cytopenias shows that there is some mechanism of caused by the CAR T-cells that leads to myelosuppression.[31] Neutropenia

is the most common type, affecting more than 81%-94% of patients (Table 2).[21,28,29] Thrombocytopenia is the second most common type, affecting between 54%-80% and anemia affecting 51%-66% (Table 2).[21,28,29] Cytopenias are typically managed by administering growth factors or through transfusions.[21,31] However, what is possibly more concerning are prolonged cytopenias.

Table 3. Frequency and Duration of Cytopenias after FDA Approved CD19 CAR-T Products

	Neutropenia ≥ Grade 3			Anemia ≥ Grade 3			Thrombocytopenia ≥ Grade 3		
	Percentage of Patients at Any Time*	At ≥ 28 Days†	At ≥ 3 Months	Percentage of Patients at Any Time*	At ≥ 28 Days†	At ≥ 3 Months	Percentage of Patients at Any Time*	At ≥ 28 Days†	At ≥ 3 Months
Axicabtagene ciloleucel, ZUMA-1 [4],*	93	26	11	66	10	3	58	24	7
Tisagenlecleucel, JULIET [2],*	81	24	0	58	Not reported	Not reported	54	41	38

* Data from package insert, all other data from studies.

† ≥30 day outcomes reported for ZUMA-1, ≥28 day outcomes reported for JULIET.

Data regarding frequency of cytopenias from the two studies that allowed for FDA approval of CAR T-cell therapy in aggressive B-cell NHL: ZUMA-1 and JULIET. Taken from Jain T, Bar M, Kansagra AJ, et al, 2019

The definition for prolonged cytopenias differs slightly in regard to time since infusion, but it generally refers to cytopenias lasting more than 28-42 days post-infusion.[37–39] Data from the ZUMA-1 study and the JULIET study show the rates of prolonged cytopenias respectively being 26% and 24% for neutropenia, 24% and 41% for thrombopenia, and 10% for anemia in the ZUMA-1 study with no data for prolonged anemia in the JULIET study (Table 2).[21,28,29] Prolonged cytopenias have been associated with many factors including CRS severity, tumor burden, number of prior therapies, and prior HD-ASCT or allogenic transplants.[21]

One study conducted by Nahas and colleagues sought out to identify potential risk factors associated with prolonged cytopenias.[38] They investigated the number of prior chemotherapy regimens, ANC and PLT counts on the first day of lymphodepletion, CRS grade 3 and above, baseline CRP, ferritin and CRP peak after infusion, and time from infusion to maximum grade CRS as possible predictive factors.[38] They reported two significant findings: a thrombocytopenia of ≤ 75,000/microliter on the first day of lymphodepletion and the median time of less than one day to reach maximum CRS post-infusion.[38] It is worth noting that this study included the analysis of twenty-two patients, of which only eight experienced prolonged cytopenias.[38]

SPECIFIC AIMS

- Identify clinical predictors along with pretreatment patient and disease precursors that increase risk of experiencing prolonged cytopenias after CAR T-cell therapy.
- Analyze the difference in patients with and without prolonged cytopenias in regard to response efficacy and rate and severity of other toxicities from CAR T-cell therapy.

METHODS

Study Population and Eligibility

This study was a retrospective analysis of patients who received commercial CAR T-cell therapy at DFCI, either axicabtagene ciloleucel or tisagenlecleucel, from December 2017 to September 2019. Protocols that allow for retrospective analysis were approved by DFCI's Institutional Review Board (IRB). There were 106 commercial CAR T-cell recipients consented to these protocols during this period of time. More specific criteria were used to delineate the portion of this population that experienced prolonged cytopenias.

For our prolonged cytopenic cohort, we required that the patients must be alive at least three months post-infusion and have blood work for at least two of the following three timepoints: one-, three-, or six-months post-infusion. We examined Hgb, ANC, and PLT counts for one month, three months, and six months post-infusion, as available per each patient. We considered cytopenic values as Hgb <10 g/dL, grade 2 per CTCAE criteria, PLT < 50,000/mm3, and ANC <1000/mm3, both grade 3 per CTCAE criteria (Table 2).[36] For a patients to be considered to have prolonged cytopenias, they must either have more than 1 time point post-infusion where cytopenic values were recorded for the same type of cytopenia(s). If they only have one timepoint where a cytopenic value was recorded, they must have received a transfusion of red blood cells (RBC) or platelets or received granulocyte-colony stimulating factor (GCSF) after their last cytopenic value since this could be the cause for the recovery of their cytopenic counts. Figure 1 visually

shows the schema for selecting our cytopenic population. These criteria identified 22 patients that matched our definition of having prolonged cytopenias.

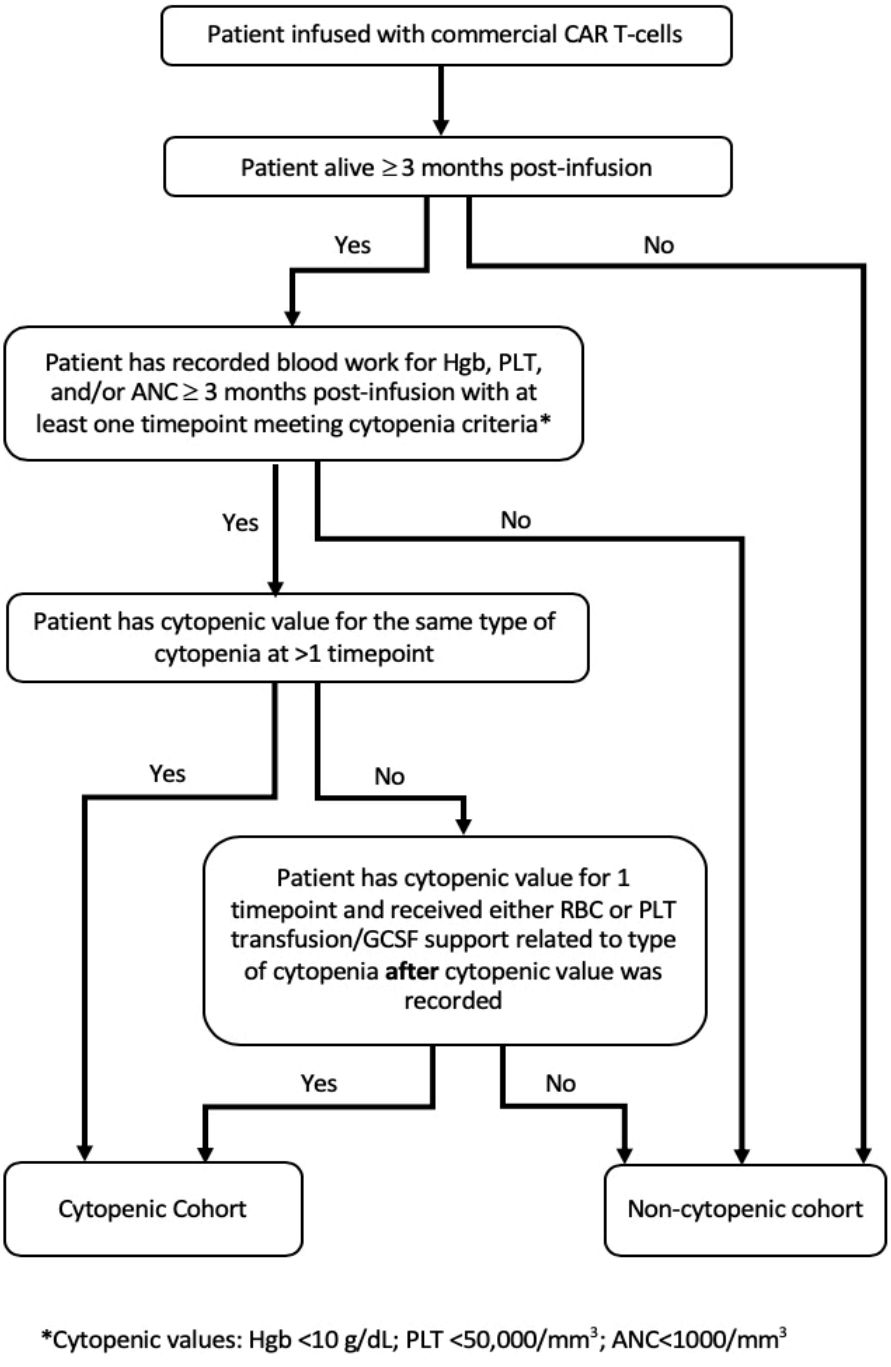

Figure 1: Patient Selection Criteria Flow Chart

Falling outside the criteria for prolonged cytopenias were 84 patients. The requirement for being considered part of the non-cytopenic, commercial CAR T-cell cohort was that the patients must have been infused with commercial CAR T-cells. There was no requirement for specific timepoints of blood work or length of time alive post-infusion.

Data Collection

Through retrospective review of patient medical records, we recorded data involving potential predictors of prolonged cytopenias, incidences of AE and if they required intervention, response rates to therapy from PET/CT scans, and various laboratory values, mainly collected from blood samples, from pre- and post-infusion. A full list of variables collected can be found in Appendix I. The Eastern Cooperative Oncology Group (ECOG) Performance Status was used to note a patient's level of function before undergoing CAR T-cell therapy. To examine if prognostic values had any relation to experiencing prolonged cytopenias, the International Prognostic Index (IPI) was collected for patients pre-lymphodepletion. In regard to PET/CT scans, the Deauville criteria and the Lugano staging and response classifications were used to determine status of disease prior to CAR T-cell therapy as well as the response post-infusion. CRS was graded based on the Lee criteria and ICANS was graded based on CTCAE criteria. All of the aforementioned grading methods' criteria can be found in Appendix II.

In order to consider additional factors involved when a patient has AEs, we also collected data for tocilizumab and steroid administration during the patients' hospital

admissions. We noted if the patient received any of these medications and then further grouped patients by the number of doses received. For analysis of tocilizumab, we separated patients into two groups: those that had only one dose and those who had two or more. For steroids, we also had two groups: patients who had four or less doses of steroids and patients who had five or more.

Statistical Analysis

Patient characteristics were reported with summary statistics for the overall cohort and by group (cytopenic versus non-cytopenic). Continuous, nominal, and ordinal variables were tested for association by group using Wilcoxon rank-sum, Fisher's exact, and Kruskal-Wallis trend tests, respectively. Patient responses were summarized as proportions with 95% exact binomial confidence intervals (CI). Overall survival (OS) was measured from date of infusion until death from any cause; patients alive at data collection were censored at last contact for OS. Progression-free survival was measured from date of infusion until progression or death from any cause; patients alive and progression-free at data collection were censored at last contact for PFS. Survival distributions by group were estimated using the Kaplan-Meier method, and differences between groups were assessed with log-rank tests. Uni- and multi-variable logistic regressions were performed using Firth's bias-reduced penalized likelihood method due to a small number of cytopenic patients. Logistic models were summarized as odds ratios (OR) with CIs and p-values based on the penalized log-likelihood. Multivariable model selection was performed using a forward/backward stepwise variable selection procedure

based on a penalized likelihood ratio test. Naïve and corrected p-values for univariable analyses were calculated; where indicated p-value adjustments for multiple tested was performed using the Benjamini-Hochberg method. All analyses were performed using R v 3.6.0 (R Core Team) with *survival* (v 3.1.8) and *logistf* (v 1.23) packages.

RESULTS

Patient Characteristics and Outcomes

There were 106 patients who received commercial CAR T-cell therapy included in the study. The median age was 62 (range: 19 – 80) years old. 67 patients had de novo aggressive B-cell NHL while 39 had transformed aggressive B-cell NHL. 32% had previously been treated with HD-ASCT. The median number of prior treatment regimens was 3 (range: 2 - 9). The median time with aggressive B-cell NHL until day of infusion was 11.6 months. Additionally, the median time since last chemotherapy regimen before CAR T-cell infusion was 3.1 months. 81% (95% CI: 72 – 88%) of patients achieved and overall response rate of either CR or partial response (PR), while 19% had a best overall response of either stable disease (SD) or progressive disease (PD). In terms of best CR rate, 64 (60%, 95% CI: 50 – 70%) patients achieved CR while 42 had the best response of either PR, SD, or PD. Data regarding the aforementioned patient characteristics is presented in table 4 and is further separated into cytopenic and non-cytopenic cohorts.

The length of time from diagnosis of aggressive B-cell NHL to date of CAR T-cell infusion was found to be positively corelated with an increased risk of developing prolonged cytopenias after CAR T-cell therapy. For the cytopenic population, the median time from diagnosis of aggressive B-cell lymphoma to CAR T-cell infusion was 15.2 months, whereas the non-cytopenic population's median time was 10.3 months (Table 4).

Table 4. Patient Characteristics

	Total n = 106 (%)	Cohort		*p-value*
		Cytopenic n = 22 (21)	Non-cytopenic n = 84 (79)	
Age at infusion				
Median (range)	62 (19 - 80)	64 (43 - 73)	62 (19 - 80)	*0.71*†
Diagnosis				
Transformed	39 (37)	9 (41)	30 (36)	*0.80*‡
de novo	67 (63)	13 (59)	54 (64)	
Stage at diagnosis				
1	14 (13)	2 (9)	12 (14)	*0.63*§
2	10 (9)	2 (9)	8 (10)	
3	33 (31)	10 (45)	23 (27)	
4	49 (46)	8 (36)	41 (49)	
ECOG				
0	38 (36)	6 (27)	32 (38)	*0.29*§
1	54 (51)	11 (50)	43 (51)	
2	8 (8)	2 (9)	6 (7)	
3	1 (1)	1 (5)	-	
Missing	*5 (5)*	*2 (9)*	*3 (4)*	
IPI (pre-lymphodepletion)				
0	9 (8)	1 (5)	8 (10)	*0.74*§
1	20 (19)	4 (18)	16 (19)	
2	26 (25)	8 (36)	18 (21)	
3	25 (24)	4 (18)	21 (25)	
4	17 (16)	2 (9)	15 (18)	
5	3 (3)	1 (5)	2 (2)	
Missing	*6 (6)*	*2 (9)*	*4 (5)*	
Prior auto transplant				
Yes	34 (32)	7 (32)	27 (32)	*> 0.99*‡
No	72 (68)	15 (68)	57 (68)	
Number of prior treatment regimens				
Median (range)	3 (2 - 9)	3 (2 - 9)	3 (2 - 8)	*0.56*‡
Bone Marrow Involvement				
Yes	15 (14)	3 (14)	12 (14)	*> 0.99*‡
No	91 (86)	19 (86)	72 (86)	

Table 4. Patient Characteristics (continued)

	Total	Cohort		*p-value*
	n = 106 (%)	**Cytopenic n = 22 (21)**	**Non-cytopenic n = 84 (79)**	
Time with aggressive lymphoma diagnosis (months)				
Median (range)	11.6 (3.7 - 25.8)	15.2 (8.0 - 24.5)	10.3 (3.7 - 25.8)	*0.011*†
Time since last chemotherapy before CAR T-cell infusion (months)				
Median (range)	3.1 (1.1 - 197.5)	3.7 (1.4 - 197.5)	3.0 (1.1 - 139.5)	*0.73*†
Time between leukapheresis and infusion (days)				
Median (range)	26 (5 - 70)	27 (22 - 53)	26 (5 - 70)	*0.29*†
Best overall response rate				
CR/PR	86 (81)	21 (95)	65 (77)	*0.067*‡
SD/PD	20 (19)	1 (5)	19 (23)	
Best complete response rate				
CR	64 (60)	15 (68)	49 (58)	*0.47*‡
PR/SD/PD	42 (40)	7 (32)	35 (42)	

†Wilcoxon rank-sum test, ‡Fisher's exact test, §Kruskal-Wallis trend test

Cytopenic Population Characteristics

Of the 22 patients in the cytopenic cohort, 8 had only one type of cytopenia, 9 had two types, and 5 had all three types of cytopenia (Figure 2). In total, there were 16 patients with prolonged anemia, 13 patients with prolonged thrombocytopenia, and 12 patients with neutropenia (Figure 3). For the 9 patients that had two types of cytopenias, 5 has anemia and thrombocytopenia, 2 had neutropenia and thrombocytopenia, and 2 had anemia and neutropenia (Figure 4).

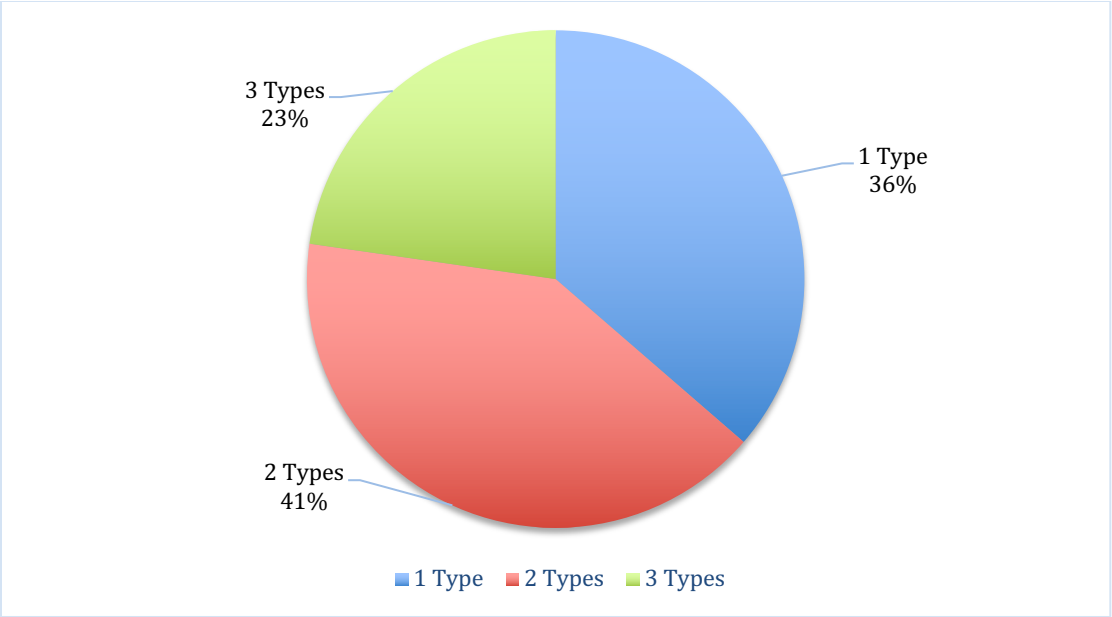

Figure 2: Number of Prolonged Cytopenias Experienced by Each Patient in Cytopenic Cohort

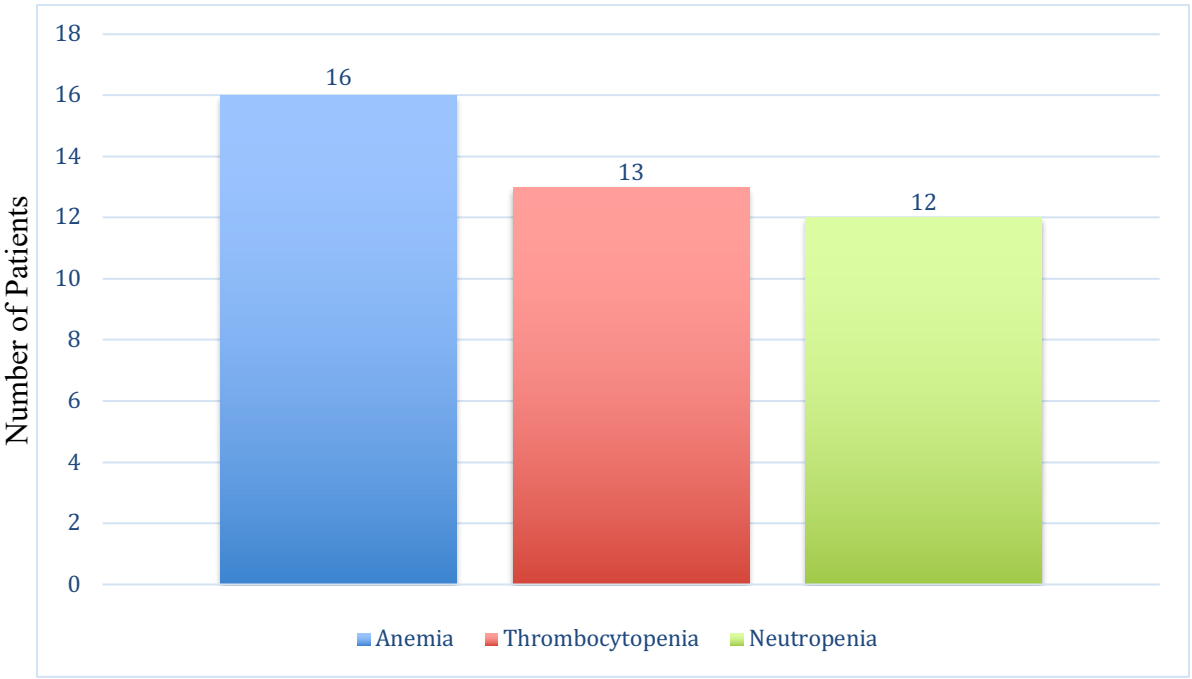

Figure 3: Frequency of Each Prolonged Cytopenia in Cytopenic Cohort

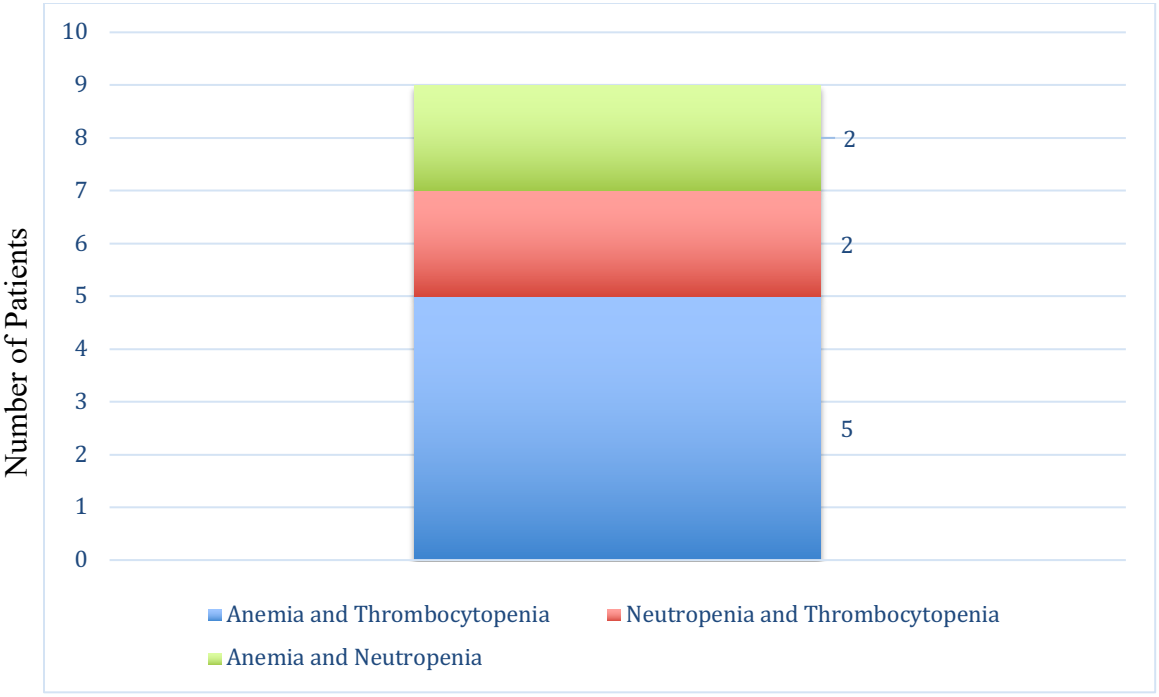

Figure 4: Frequency of Cytopenia Combinations in Patients with Two Types of Prolonged Cytopenias

Four of the 22 patients underwent radiation therapy before leukapheresis for CAR T-cell therapy. Of these four patients, one had radiation to the left upper quadrant of the abdomen, one to the mediastinum, and two to the breast. Furthermore, seven patients had bone marrow biopsies done post-infusion of CAR T-cell. One patient was found to have Myelodysplastic Syndrome (MDS) (Table 5). All patients were found to have hypocellular marrow and furthermore, all had decreased myeloid cell lines (Table 5). 5 of the 7 patients had decreased megakaryocyte cell lines while the other 2 patients' megakaryocyte cell lines were normal (Table 5). The most variation was seen in the erythroid cell line with 3 patients having an increase, 3 having a decrease, and one patient having an expected proportion due to them having MDS (Table 5).

Table 5. Post-Infusion Bone Marrow Biopsy Results

Patient	MDS	Comments on Cellularity	Comments on erythroid cell line	Comments on myeloid cell line	Comments on megakaryocyte cell line	Comments on blasts	Cytogenetics
24	N	decreased	increased; hyperplasia	decreased	decreased		N/A
28	N	hypo / dilute;	moderately increased	rare and left shifted maturation	moderately decreased	<5% of marrow cellularity	N/A
29	N	decreased	decreased	decreased and left-shifted maturation	moderately decreased		XY hybridization pattern in 100% of cells
42	Y	decreased	moderately proportional and maturation with occasional dysplastic forms	moderately decreased and left shifted maturation	moderately decreased and frequent dysplastic forms		7q deletion
52	N	decreased	mildly proportionally increased	mildly proportionally decreased	normal		51-78<3n>,XX,-Y,+2,+4,+6,add(8)(p23)x2,add(10)(p?12)x2,-13,-14,add(14)(q32),
66	N	increased. Spicules present	markedly proportionally decreased and left shifted	markedly decreased and left-shifted	normal		N/A
67	N	moderately Hypercellular with subtotal necrosis	markedly decreases	markedly decreased	rare		N/A

MDS: Myelodysplastic Syndrome

Chemotherapy Regimens

Although we found that the number of prior lines of therapy a patient had received prior to CAR T-cell infusion was not a predictive factor of prolonged cytopenias, we further investigated to see if any individual regimens were predictive. To explore this, we analyzed the rate of all chemotherapy used throughout the course of a patient's treatment as well as what chemotherapy regimens were used prior to CAR T-cell infusion.

When investigating all chemotherapy regimens administered prior to apheresis, the two most common in both cohorts were RCHOP and RICE (Table 6). This is not surprising considering RCHOP is the standard first line therapy for patients with aggressive B-cell NHL and RICE is a common salvage therapy option. Both RICE and lenalidomide were found to be administered at higher rates in the cytopenic cohort upon naïve Fisher's analysis (Table 6). However, when corrected using the BH procedure, neither of these retained their significance.

Table 6. Frequency of All Chemotherapy Regimens Before Lymphodepletion

Chemotherapy	Number of Cytopenic Patients	Number of Non-cytopenic Patients	Fisher's exact p (naïve)
R-CHOP	18*	71‡	0.75
RICE	18*	48	0.047
R-GDP	5	19§	> 0.99
BR	4	12	0.74
R-GemOx	4	7*	0.23
Lenalidomide	3	0	0.008
R-EPOCH	2	21¶	0.15
R-DHAC	2†	4	0.6
R-ESHAP	0	4*	0.58
R-CVP	2	3	0.28
R-DHAP	0	3*	> 0.99
OCHOP	0	3	> 0.99
Idelesib	1	3	> 0.99
Selinexor	0	2	> 0.99
M-BACOP	0	2	> 0.99
R-VIC	0	2	> 0.99
Venetoclax	0	2	> 0.99
Cisplatin	0	2	> 0.99

*: 1 patient did not receive rituximab; †: 1 patient also receive ifosfamide; ‡: 3 patients did not receive rituximab; §: 2 patients did not receive rituximab; ¶: 4 patients received an adjusted dose;
Abbreviations: BR: bendamustine, rituximab; M-BACOP: methotrexate, bleomycin, doxorubicin, cyclophosphamide, prednisone; O-CHOP: obinutuzumab, cyclophosphamide, doxorubicin, vincristine, prednisone; R-CHOP: rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; R-CVP rituximab, cyclophosphamide, vincristine, prednisone; R-cytarabine: rituximab, cytarabine; R-DHAC rituximab, dexamethasone, cytarabine, carboplatin; R-DHAP rituximab, dexamethasone, cytarabine, cisplatin; R-EPOCH rituximab, etoposide, prednisone, vincristine, cyclophosphamide, docorubicin; R-ESHAP rituximab, etoposide, solu-medrone, cytarabine, cisplatin; R-GDP: rituximab, gemcitabine, dexamethasone, cisplatin; R-gem/navelbine: rituximab, gemcitabine, navelbine; R-GemOx: rituximab, gemcitabine, oxaliplatin; RICE: rituximab, ifosfamide, carboplatin, etoposide; R-VIC: rituximab, etoposide, ifosfamide, carboplatin
Includes any chemotherapy regimen administered to at least 2 patients in at least one of the cohorts.

We examined the last chemotherapy regimens used before apheresis. RICE was the most common in both cohorts (Table 7). Out of all the regimens analyzed, R-cytarabine was the only one to be associated with an increased risk of prolonged cytopenias based on a naïve Fisher's exact test (Table 7). However, this finding did not hold up to BH correction.

Table 7. Frequency of Last Used Chemotherapy Regimens Before Lymphodepletion

Chemotherapy Regimen	Cytopenic	Non-Cytopenic	Fisher's exact p (naïve)
RICE	8*	28	0.8
R-GDP	3	8	0.69
BEAM	3	10	0.73
R-GemOx	2	8*	> 0.99
R-cytarabine	2	0	0.042
R-GDC	1	2*	0.51
R-CHOP	1	2	0.51
BR	1	2†	0.51
R-gem/navelbine	1	0	0.21
R-DHAP	0	3*	> 0.99
R-EPOCH	0	3‡	> 0.99
R-DHAC	0	3	> 0.99
O-CHOP	0	2§	> 0.99
Unlituximab, bendamustine, umbralisib	0	2	> 0.99
Selinexor clinical trial	0	2	> 0.99

*: 1 patient received regimen without rituximab (R); †: 2 patients received regimen with polatuzumab; and ibrutinib with regimen, ‡: 1 patient received regimen at an adjusted dose; §: 1 patient received venetoclax

Abbreviations: BEAM: carmustine, etoposide, cytarabine, melphalan; BR: bendamustine, rituximab; O-CHOP: obinutuzumab, cyclophosphamide, doxorubicin, vincristine, prednisone; R-CHOP: rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; R-cytarabine: rituximab, cytarabine; R-DHAC rituximab, dexamethasone, cytarabine, carboplatin; R-DHAP rituximab, dexamethasone, cytarabine, cisplatin; R-EPOCH rituximab, etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin; R-GDP: rituximab, gemcitabine, dexamethasone, cisplatin; R-gem/navelbine: rituximab, gemcitabine, navelbine; R-GemOx: rituximab, gemcitabine, oxaliplatin; RICE: rituximab, ifosfamide, carboplatin, etoposide; R-VIC: rituximab, etoposide, ifosfamide, carboplatin

Includes any chemotherapy regimen administered to at least 2 patients in at least one of the cohorts.

Laboratory Results

We collected data on eight different laboratory tests of interest at varying time points totaling to 19 different values of interest (Table 8). Of these 19, lower values of ANC on day 0, PLT on day 0, and Hgb pre-apheresis and on day 0 were all associated with an increased risk of prolonged cytopenia (Table 8). Additionally, higher values of CRP pre-lymphodepletion and on day 0 were associated with an increased risk of prolonged cytopenia (Table 8).

Table 8. Laboratory Results

	Total n = 106 (%)	Cohort Cytopenic n = 22 (21)	Cohort Non-cytopenic n = 84 (79)	*p-value*
ANC pre-leukapheresis				
Median (range)	3560 (460 - 71600)	2895 (1420 - 6240)	3780 (460 - 71600)	*0.077*†
Missing	*2 (2)*	*0 (0)*	*2 (2)*	
ANC day 0				
Median (range)	3485 (90 - 99000)	1530 (290 - 6890)	3960 (90 - 99000)	*< 0.001*†
Missing	*4 (4)*	*1 (5)*	*3 (4)*	
CRP pre-lymphodepletion				
Median (range)	10.1 (0.3 - 190.6)	17.8 (1.0 - 190.6)	8.4 (0.3 - 187.3)	*0.034*†
Missing	*2 (2)*	*2 (9)*	*0 (0)*	
CRP day 0				
Median (range)	21.2 (2.8 - 300.0)	41.1 (7.6 - 300.0)	18.5 (2.8 - 300.0)	*0.045*†
Missing	*4 (4)*	*0 (0)*	*4 (5)*	
CRP max post-CARs				
Median (range)	98.5 (4.8 - 300.0)	141.6 (28.9 - 300.0)	91.5 (4.8 - 300.0)	*0.12*†
LDH pre-lymphodepletion				
Median (range)	212 (85 - 1722)	258 (85 - 959)	208 (109 - 1722)	*0.44*†
LDH day 0				
Median (range)	242.0 (109.5 - 2150.0)	280.0 (170.0 - 1931.0)	236.0 (109.5 - 2150.0)	*0.16*†
Missing	*1 (1)*	*0 (0)*	*1 (1)*	

Table 8. Laboratory Results (continued)

	Total n = 106 (%)	Cohort		*p-value*
		Cytopenic n = 22 (21)	Non-cytopenic n = 84 (79)	
Ferritin pre-lymphodepletion				
Median (range)	851 (107 - 7965)	888 (267 - 3466)	776 (107 - 7965)	*0.33*†
Missing	*81 (76)*	*13 (59)*	*68 (81)*	
Ferritin day 0				
Median (range)	716 (75 - 20003)	1205 (249 - 7846)	667 (75 - 20003)	*0.076*†
Missing	*6 (6)*	*1 (5)*	*5 (6)*	
Ferritin max post-CARs				
Median (range)	1612 (48 - 61104)	2321 (476 - 27505)	1482 (48 - 61104)	*0.097*†
Missing	*9 (8)*	*1 (5)*	*8 (10)*	
ALC at leukapheresis				
Median (range)	595 (90 - 7600)	530 (90 - 1880)	605 (90 - 7600)	*0.67*†
ALC max post-CARs				
Median (range)	720 (20 - 12650)	710 (100 - 3780)	735 (20 - 12650)	*0.80*†
ALC at max grade CRS				
Median (range)	70 (0 - 11330)	60 (10 - 1180)	80 (0 - 11330)	*0.79*†
Missing	*36 (34)*	*7 (32)*	*29 (35)*	
IL-6 day 0				
Median (range)	4.9 (1.2 - 88.6)	8.1 (1.3 - 43.6)	4.8 (1.2 - 88.6)	*0.29*†
Missing	*27 (25)*	*7 (32)*	*20 (24)*	
IL-6 max				
Median (range)	160.0 (5.7 - 400.0)	400.0 (5.9 - 400.0)	113.5 (5.7 - 400.0)	*0.065*†
Missing	*25 (24)*	*7 (32)*	*18 (21)*	
Platelets pre-leukapheresis				
Median (range)	164 (28 - 503)	126 (28 - 421)	171 (40 - 503)	*0.079*†
Missing	*2 (2)*	*0 (0)*	*2 (2)*	
Platelets day 0				
Median (range)	99 (7 - 311)	60 (7 - 311)	105 (12 - 305)	*0.007*†
Missing	*2 (2)*	*0 (0)*	*2 (2)*	

Table 8. Laboratory Results (continued)

	Total	Cohort		*p-value*
	n = 106 (%)	Cytopenic n = 22 (21)	Non-cytopenic n = 84 (79)	
Hemoglobin pre-leukapheresis				
Median (range)	10.6 (6.6 - 14.5)	9.8 (6.6 - 12.6)	11.1 (7.2 - 14.5)	*0.006*†
Missing	*2 (2)*	*0 (0)*	*2 (2)*	
Hemoglobin day 0				
Median (range)	9.50 (3.11 - 13.30)	8.65 (6.20 - 11.60)	9.90 (3.11 - 13.30)	*0.005*†
Missing	*2 (2)*	*0 (0)*	*2 (2)*	

†Wilcoxon rank-sum test

Abbreviations: ANC: absolute neutrophil count; CRP: C-reactive protein; LDH: lactate dehydrogenase; ALC: absolute lymphocyte count; IL-6: interleukin-6

To further understand which of these variables are most associated with increased risk of prolonged cytopenias, we performed an additional univariate analysis to the results of Table 8. This allowed us to not only see which values correlated with either cohort, but also the degree of association the individual results had with the cohorts. This analysis determined that higher values for Hgb pre-leukapheresis and on day 0 are associated with not having prolonged cytopenias (Figure 5).

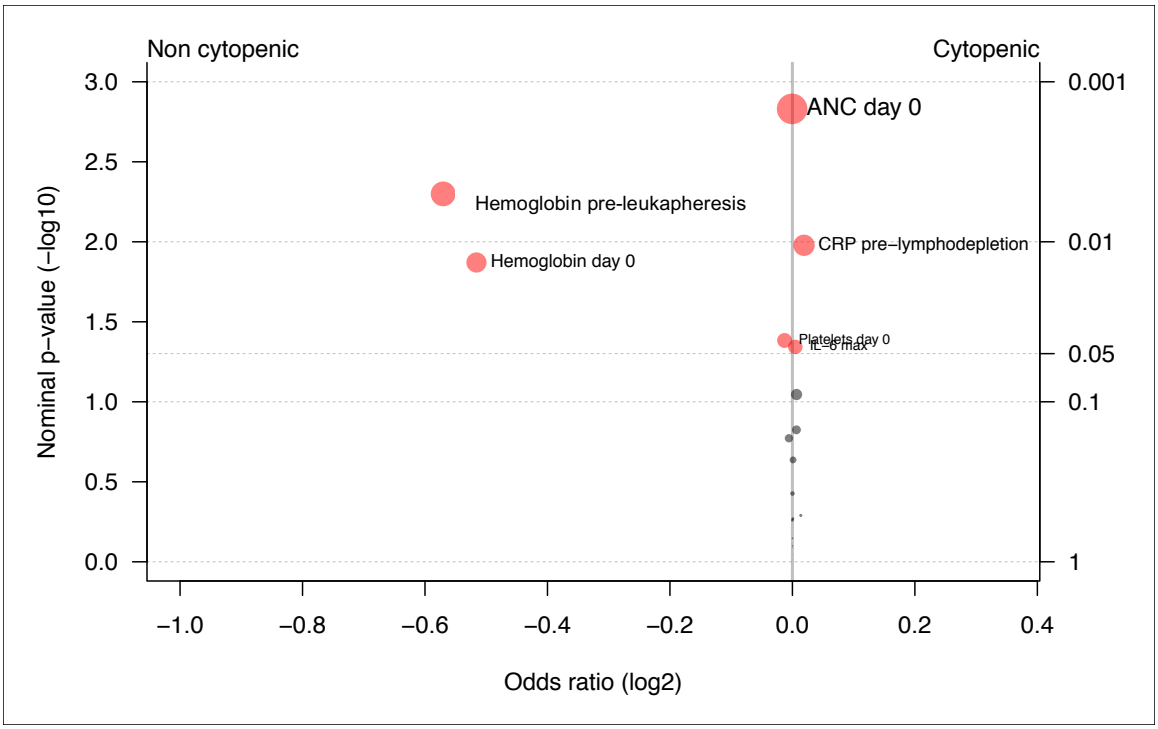

Figure 5: Volcano Plot of Laboratory Values

Abbreviations: ANC: absolute neutrophil count; CRP: C-reactive protein; IL-6: interleukin-6

Visual representation of univariate analysis of laboratory values. Data points with a negative odds ratio represents that higher value of those laboratory test are associated with non-cytopenic patients. Conversely, data points with a positive odds ratio are associated with having higher values being found in cytopenic patients.

Furthermore, a multivariable analysis was also conducted on the laboratory values to determine which values maintained their significance in comparison to the other values. Three values were determined to remain significant (Figure 6). Cytopenic patients had significantly higher CRP values pre-lymphodepletion than non-cytopenic patients (Figure 6). Meanwhile, patients without prolonged cytopenias were more likely to have a higher ferritin and PLT values on day 0 (Figure 6).

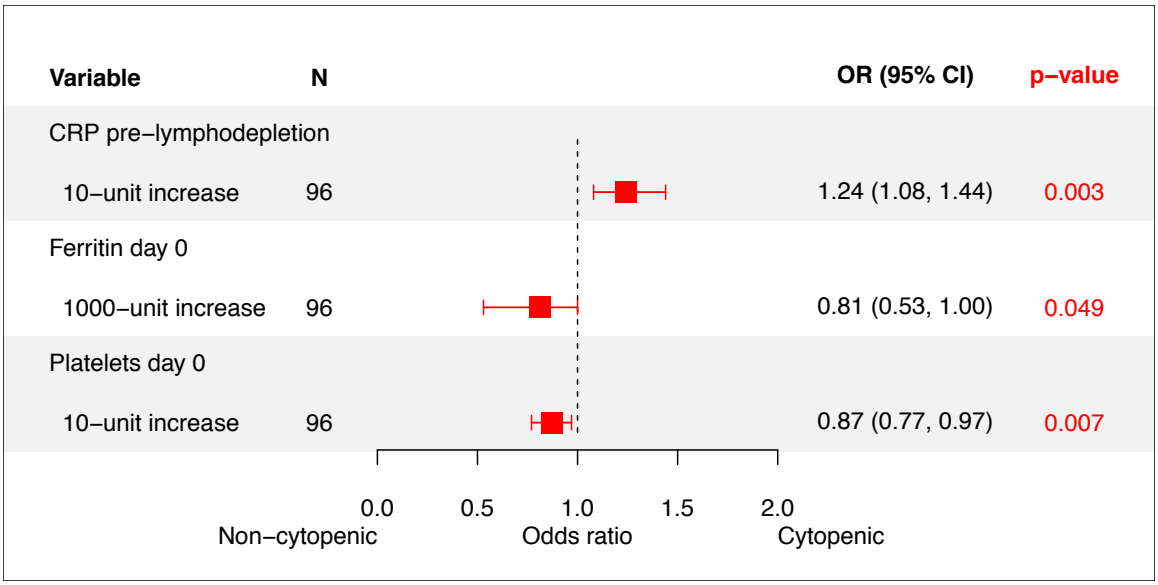

Figure 6: Forest Plot of Laboratory Values

Abbreviation: CRP: C-reactive protein. Visual representation of the multivariate analysis of laboratory values. Odds ratio of less than one suggests higher laboratory values are more likely to be observed in non-cytopenic patients. Meanwhile, odds ratio of greater than one would suggest higher laboratory values would be found in cytopenic patients.

Adverse Events and Related Interventions

87% of all patients in the study experienced CRS with 8% having max grade 3 or higher (Table 9). Moreover, 45% of patients had max grade 2 CRS and 34% had max grade 1 CRS (Table 9). The median duration of CRS was 6 days with time to max CRS being 4 days (Table 9). The distribution of max CRS grade for cytopenic and non-cytopenic patients was not statistically different (Figure 7). Furthermore, 61% of all patients had some severity of ICANS with 34% having grade 3 or higher (Table 9). Similar to CRS, the distribution of max ICANS grading was not statistically different between the cytopenic and non-cytopenic cohorts (Figure 8). When comparing cytopenic and non-cytopenic cohorts in regards of incidence of CRS and ICANS, there was no statistically significant association with either cohort (Table 9).

Table 9. Adverse Events Prevalence, Severity, and Duration

	Total n = 106 (%)	Cohort		*p-value*
		Cytopenic n = 22 (21)	Non-cytopenic n = 84 (79)	
Max grade CRS				
0	13 (12)	2 (9)	11 (13)	*0.50*§
1	36 (34)	7 (32)	29 (35)	
2	48 (45)	11 (50)	37 (44)	
3	4 (4)	1 (5)	3 (4)	
4	3 (3)	1 (5)	2 (2)	
5	1 (1)	-	1 (1)	
Missing	*1 (1)*	*0 (0)*	*1 (1)*	
CRS grade ≥ 2				
Yes	56 (53)	13 (59)	43 (51)	*0.63*‡
No	49 (46)	9 (41)	40 (48)	
Missing	*1 (1)*	*0 (0)*	*1 (1)*	
Duration of CRS (any grade)				
Median (range)	6 (0 - 20)	6 (0 - 15)	6 (0 - 20)	*0.40*†
Missing	*12 (11)*	*1 (5)*	*11 (13)*	
Time to max CRS				
Median (range)	4 (0 - 12)	4 (0 - 10)	4 (0 - 12)	*0.21*†
Missing	*14 (13)*	*2 (9)*	*12 (14)*	
Time to grade 2+ CRS				
Median (range)	4 (0 - 12)	4 (0 - 10)	4 (0 - 12)	*0.41*†
Missing	*50 (47)*	*9 (41)*	*41 (49)*	
Max grade ICANS				
0	40 (38)	6 (27)	34 (40)	*0.49*§
1	16 (15)	4 (18)	12 (14)	
2	13 (12)	4 (18)	9 (11)	
3	12 (11)	3 (14)	9 (11)	
3a	19 (18)	5 (23)	14 (17)	
3b	5 (5)	-	5 (6)	
Missing	*1 (1)*	*0 (0)*	*1 (1)*	
ICANS grade ≥ 2				
Yes	49 (46)	12 (55)	37 (44)	*0.47*‡
No	56 (53)	10 (45)	46 (55)	
Missing	*1 (1)*	*0 (0)*	*1 (1)*	

§Kruskal-Wallis trend test, ‡Fisher's exact test, †Wilcoxon rank-sum test

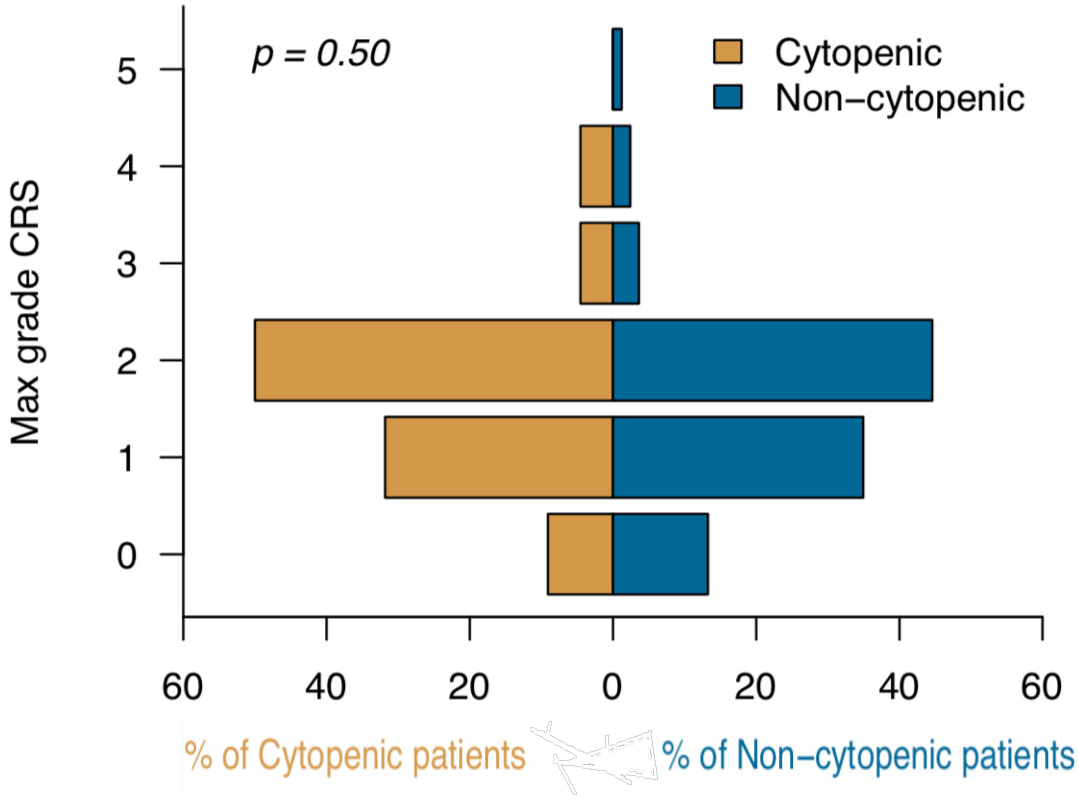

Figure 7: Distribution of CRS in Cytopenic and Non-cytopenic Cohorts

Abbreviation: CRS: Cytokine Release Syndrome

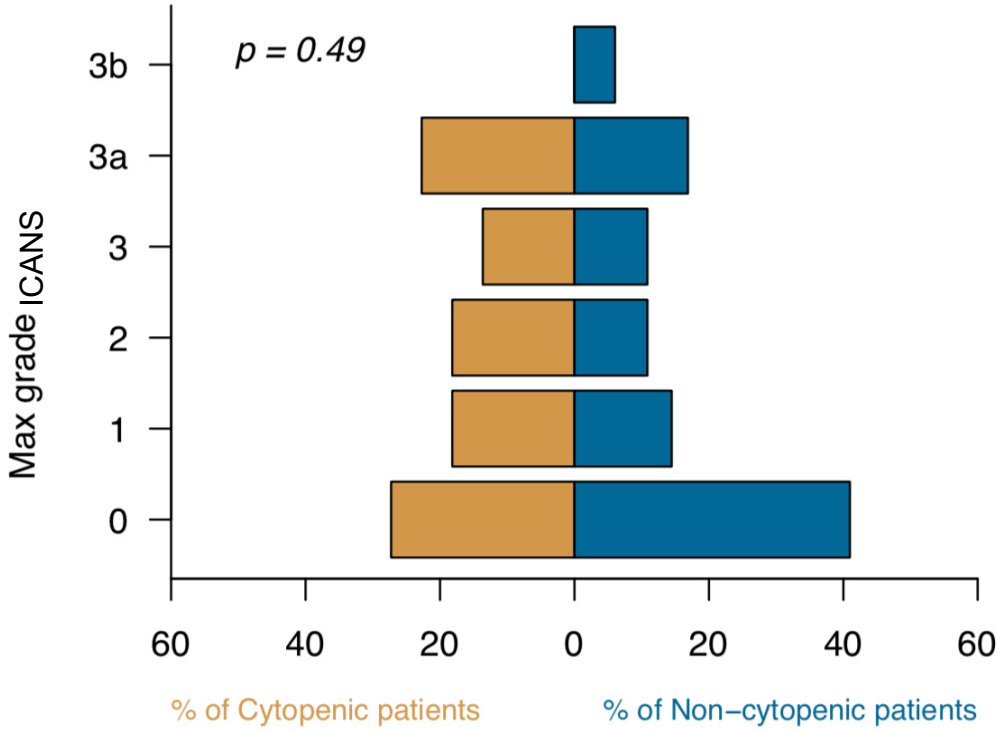

Figure 8: Distribution of ICANS in Cytopenic and Non-cytopenic Cohorts

Abbreviation: ICANS: IEC-Associated Neurotoxicity Syndrome

When examining tocilizumab and steroid administration, we found that 68 patients received tocilizumab. Of those 68 patients, 26 received one dose of tocilizumab while 42 patients were given two or more doses. Steroids were administered for CRS management to 40 patients while they were administered for ICANS management to 41 patients. When considering total steroid administration, 26 patients received four or less doses of steroids, whereas 30 patients received five or more doses. No statistical significance was found regarding differences in tocilizumab or steroid administration between cytopenic and non-cytopenic cohorts. Data regarding tocilizumab and steroid administration can be found in table 10.

Table 10. Tocilizumab and Steroid Administration for CRS and ICANS

		Cohort		
	Total n = 106 (%)	**Cytopenic** n = 22 (21)	**Non-cytopenic** n = 84 (79)	***p-value***
Tocilizumab administered				
Yes	68 (64)	16 (73)	52 (62)	*0.46*‡
No	38 (36)	6 (27)	32 (38)	
Steroids administered for CRS				
Yes	40 (38)	7 (32)	33 (39)	*0.62*‡
No	66 (62)	15 (68)	51 (61)	
Steroids administered for ICANS				
Yes	41 (39)	10 (45)	31 (37)	*0.47*‡
No	65 (61)	12 (55)	53 (63)	
Doses of tocilizumab				
2+	42 (40)	12 (55)	30 (36)	*0.25*‡
1	26 (25)	4 (18)	22 (26)	
Missing	*38 (36)*	*6 (27)*	*32 (38)*	
Doses of steroids (for CRS or ICANS)				
5+	30 (28)	6 (27)	24 (29)	*> 0.99*‡
1-4	26 (25)	6 (27)	20 (24)	
Missing	*50 (47)*	*10 (45)*	*40 (48)*	

‡Fisher's exact test

Abbreviations: CRS: Cytokine Release Syndrome; ICANS: IEC-Associated Neurotoxicity Syndrome

Nine of the 22 patients in the cytopenic cohort experienced infections post-CAR T-cell infusion (Table 11). Thrush affected four patients, three patients had pneumonia, while upper respiratory infection (URI) and septic shock both affected two patients (Table 11). Also, several infections were only found to affect one patient each: necrotizing soft tissue infection, central line associated blood stream infection, and urinary tract infection (UTI) (Table 11). One patient passed away due to their infectious complications.

Table 11. Infections of Cytopenic Cohort

Patient #	Infection(s)
6	Septic shock, necrotizing soft tissue infection of right leg
10	Upper respiratory infection
19	Pneumonia, thrush
24	Pneumonia, thrush
29	Pneumonia
43	Central line associated blood stream infection, thrush
48	Thrush, upper respiratory infection
69	Septic shock due to MRSA pneumonia in setting of neutropenia
70	Urinary tract infection

Abbreviation: MRSA: Methicillin-resistant Staphylococcus aureus
All infections of the cytopenic cohort after receiving CAR T-cells

In the non-cytopenic cohort, 25 of the 84 patients had an infectious complication after receiving CAR T-cells (Table 12). When comparing the two cohorts, there was no difference in the incidence of infections (Table 12).

Table 12. Incidence of Infections

	# of patients who had post-infusion infection(s) within 1 year	Total # patients in cohort	Fisher's exact p-value
Cytopenic	9	22	0.32
Non-cytopenic	25	84	

Comparison of the incidence of infections between cohorts after CAR T-cell infusion

Overall and Progression-free Survival

In order to investigate if having prolonged cytopenias affect a patient's response to CAR T-cell therapy, we analyzed the PFS and OS rates of the cytopenic and non-cytopenic cohorts (Figure 9). In terms of OS, at 12 months post-infusion the cytopenic

cohort had an OS of 68% (95% CI: 48 - 97) while the non-cytopenic cohort had an OS of 64% (95% CI: 50 - 82) (Figure 9). Overall, there was no significant difference in OS between the two cohorts (Figure 9).

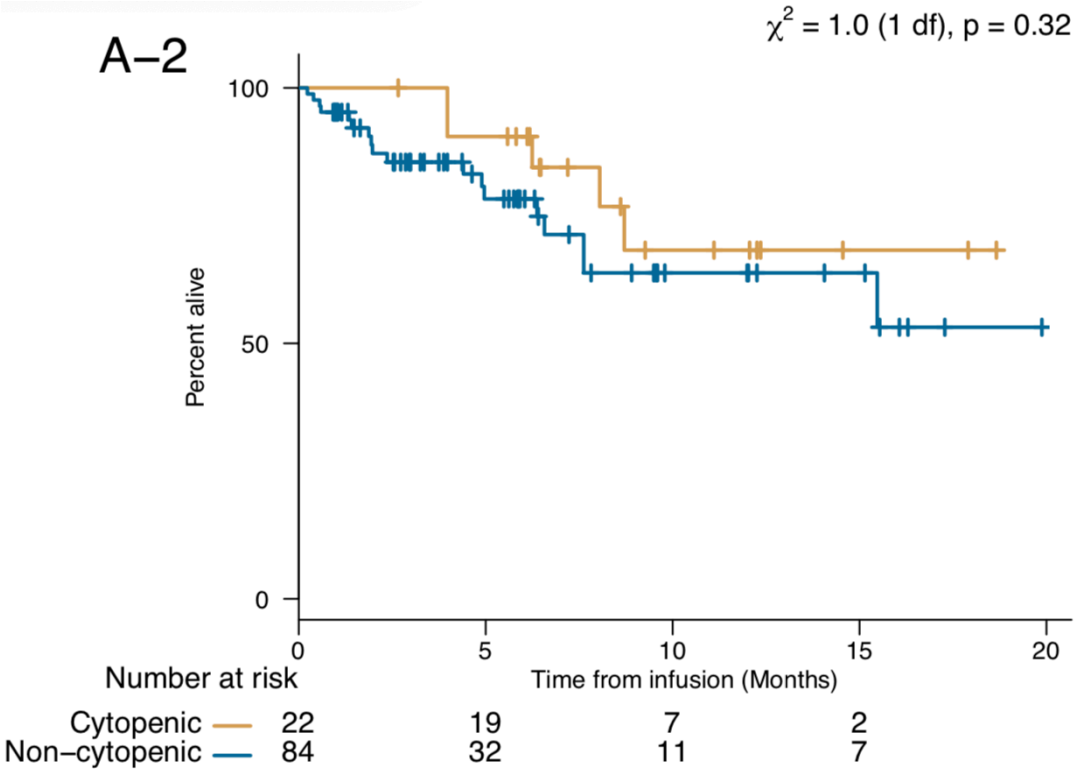

Figure 9: Overall Survival of and Cytopenic and Non-cytopenic Cohorts
Each hash mark represents a death of a patient

At 12 months the cytopenic cohort had a PFS rate of 38% (95% CI: 21 - 69) whereas the non-cytopenic cohort had a PFS rate of 46% (95% CI: 33 - 64) (Figure 10). There was no significant difference in PFS rate between the two cohorts (Figure 10).

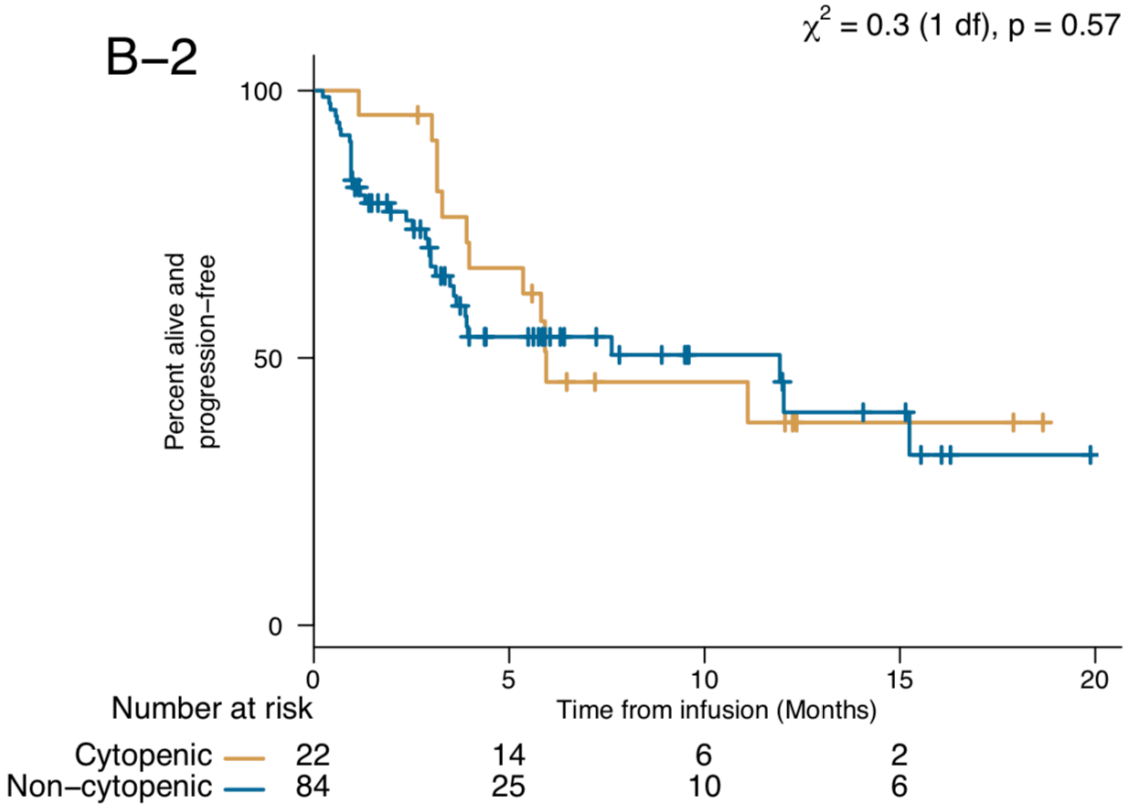

Figure 10: Progression Free Survival of Cytopenic and Non-cytopenic Cohorts
Each hash mark represents either a progression of disease or death of a patient.

DISCUSSION

CAR T-cell therapy has given patients who formerly had few effective salvage treatment options an option that could potentially prove curative. However, CAR T-cell therapy comes with its own challenges including many well documented toxicities. While CRS and ICANS have been the focus of most investigations, there is a lack of understanding around hematologic toxicities. This study set out to identify possible clinical predictors and patient and disease precursors of prolonged cytopenias in order to better identify patients who are at risk of developing prolonged cytopenias.

Through this retrospective analysis of patients infused with commercial CAR T-cells, we found several statistically significant differences between the cytopenic and non-cytopenic cohort. The first of these being the length of time with an aggressive B-cell NHL diagnosis in relation to the day of CAR T-cell infusion. A longer time between diagnosis and CAR T-cell infusion was found to be associated with prolonged cytopenias. This is particularly interesting considering that the number of prior therapies a patient had prior to CAR T-cell therapy and if a patient had undergone an HD-ASCT were both found to not be associated with prolonged cytopenias. Due to these not providing any additional insight on why an increased length of time from diagnosis to CAR T-cell treatment is associated with prolonged cytopenias, it would be valuable for further investigations to examine what specifically about the length of time creates the association.

Our results regarding bone marrow biopsies were limited due to only having seven of the 22 cytopenic patients having had bone marrow biopsies after undergoing

CAR T-cell therapy. Furthermore, we were limited to PET/CT results for knowing if patients had disease involvement in their bone marrow before CAR T-cell therapy. It is concerning that all 7 patients did have hypocellular marrow and decrease myeloid cell lines. However, it would be beneficial to have a larger sample size with bone marrow biopsies from before and after CAR T-cell therapy to better investigate what effects CAR T-cell therapy has on a patient's bone marrow compared to prior treatments or additional factors.

Through the analysis of laboratory values, we found that low ANC, PLT, and HGB on the day of infusion of CAR T-cells as well as low Hgb before leukapheresis were all associated with prolonged cytopenias. These findings suggest that patients with less bone marrow reserve were more likely to have prolonged cytopenias. This could mean that the patients who had prolonged cytopenias may have a weakened bone marrow before CAR T-cell therapy, possibly related to prior therapies. This further supports the need to examine patients' bone marrow before and after CAR T-cell therapy.

High CRP values before lymphodepletion and on the day of CAR T-cell infusion were found to be associated with prolonged cytopenias. This could be reflective of something specific to their disease biology that could be a factor in developing prolonged cytopenias. Another possible explanation could be that their CAR T-cell expansion and/or persistence may be a factor due to higher pretreatment inflammation being related to increased CAR expansion. If this is the case, the prolonged cytopenias experienced would be an effect of CAR T-cell themselves and not of other previous treatments. Further investigation into the patients' disease cytogentics would help to understand if

specific biological aspects of aggressive B-cell NHL are associated with prolonged cytopenias.

Using multivariable analysis, high CRP values prior to lymphodepletion and on day of CAR T-cell infusion were found to be the most predictive results associated with patients experiencing prolonged cytopenias. It would be valuable for additional studies to investigate if these values are associated with prolonged cytopenias. Therefore, if found to be associated in larger population of patients, these values could be used by physicians to indicate an increased risk of a patient developing prolonged cytopenias. Conversely, high ferritin and PLT on the day of infusion were found to be associated with not having prolonged cytopenias. If further studies supported these findings, these values could be used to suggest if a patient is at lower risk of developing prolonged cytopenias. However, with this being the first study to identify this association, further research is needed before determining the predictiveness.

When examining clinical properties of the cytopenic cohort, we found that 82% of patients had been treated with RCHOP and RICE prior to CAR T-cell therapy. This is not surprising as RCHOP is the standard first line of therapy for patients with aggressive B-cell NHL and RICE is a common salvage therapy option.[8,13] However, we did find that receiving R-cytarabine as the most recent chemotherapy prior to CAR T-cell infusion was found to be associated with prolonged cytopenias through naïve Fisher exact test. Although the significance did not hold up to BH correction, it does indicate a need for further investigation to either confirm or deny any increased risk of prolonged cytopenias from receiving R-cytarabine.

We further investigated all chemotherapy regimens patients had received prior to CAR T-cell infusion and found that both RICE and lenalidomide were associated with prolonged cytopenias through naïve Fisher exact testing. However, similarly to R-cytarabine, these associations did not remain after BH correction. Therefore, they are not found to be significant, but does suggest further investigation into their association with prolonged cytopenias is warranted.

Investigations into PFS and OS were found to show no significant difference between the cytopenic and non-cytopenic cohorts. Since our criteria for the cytopenic cohort was much more defined in terms of length of data, and some of the patients in both cohorts did not have up to 6 months of follow up data, it would be of interest to further investigate these relationships with longer follow up data.

In terms of AEs, all factors involved in CRS and ICANS and the rate of infections after CAR T-cell infusion were found not to have any significant associations to either cohort. Therefore, absence or presence of these AE are not predictive of prolonged cytopenias. Considering a prior study found that time to max grade CRS was associated with prolonged cytopenias, and our research did not find this association, more studies should be conducted to further investigate this as an associated factor. [38]

Considering the lack of research regarding prolonged cytopenias after CAR T-cell infusion, it is imperative that more studies are conducted to help better understand what factors either increase a patient's risk or are predictive of a patient having prolonged cytopenias.

APPENDIX I: List of Variables Collected

Below is a list of all the variables collected and analyzed within this study.

- ID
- Patient Name
- Medical Record Number
- Date of birth
- Age at time of cell infusion
- Alive
- If deceased - date of death
- Cause of death
- Lymphoma histology (2016 WHO)
- Date of Dx (of aggressive large cell lymphoma)
- Stage at dx
- Bone Marrow Involvement at dx
- ECOG or KPS pre-CARs
- IPI pre-lymphodepletion
- # of lines of therapy pre-leukapheresis
- Prior Auto Transplant
- Date of auto transplant
- Date of relapse after auto
- Prior Allogenic Transplant
- Date of allogenic Transplant
- Date of relapse after allogenic transplant
- Name of most recent chemo regimen pre leukapheresis
- Last date of chemotherapy prior to leukapheresis
- Date of leukapheresis
- ALC on date of leukapheresis
- Date of day 1 of lymphodepletion
- Baseline CRP pre lymphodepletion
- Baseline Ferritin pre lymphodepletion
- Baseline LDH pre lymphodepletion
- G-CSF Pre-Infusion Y/N
- G-CSF type
- G-CSF date

- Date of cell infusion
- Type of Product
- Baseline CRP day 0
- Baseline Ferritin day 0
- Baseline LDH day 0
- Baseline IgG day 0
- Baseline CD4 day 0
- Day of first fever (>100.4)
- ANC on 1st day of fever (T>100.4)
- ALC on date of 1st fever post CARs
- Duration of fever (days)
- Max temperature post CARS (C)
- ALC on date of max temperature
- CRP Max post CARs
- Day of CRP Peak post CARs
- Ferritin Max post CARS
- Day of Ferritin peak post CARs (
- ALC Max post CARs
- Day of ALC peak post CARs
- Clinical CRS observed (Lee Criteria)
- Duration of CRS (days)
- Date of onset grade 2+ CRS
- Max grade CRS
- Day post CAR T infusion that max grade CRS was observed
- ALC on date of observed max grade CRS
- Was CRS self-limiting (ie - No cytokine directed therapy)
- Tocilizumab given?
- Number of doses of tocilizumab
- >1 doses of tocilizumab
- Date of first tocilizumab dose
- CRS grade at time of tocilizumab dosing
- Low dose steroids given for CRS
- Number of doses of low dose steroids given
- Evidence of ICANS
- First day of ICANS
- MAX grade ICANS
- Duration of ICANS (days)
- Low dose steroids given for ICANS

- # of doses of low dose steroids used for ICANS
- Total number of steroids >4 (From CRS and ICANS)
- Type of G-CSF during admission
- Number of doses of G-CSF during admission
- Date of initial admission to the hospital
- Date of discharge from the hospital
- Date of first restaging scan
- Time after infusion of 1st scan (days)
- Response on 1st restaging scan
- Deauville score on restaging scan
- Size of index target lesion post CARs
- ALC on date of restaging scans
- Scan #2-6 Date
- Time after infusion of scan 2-6 (days)
- Response at Scan 2-6
- Date of CR post CARs (If Achieved)
- Relapse
- Date of Relapse post CARs
- Date of restaging bone marrow biopsy
- ALC on date of restaging marrow
- Type of G-CSF post-admission
- Number of doses of G-CSF post-admission (within 6 months of infusion)
- IgG at timepoint 1-5 post-infusion
- CD4 at timepoint 1-5 post-infusion
- Timepoint 1-5 Date
- Time after infusion of timepoint 1-5 (Days)
- Length of Stay
- Hgb, PLT, and ANC Pre aph
- Hgb, PLT and ANC Day 0
- Hgb, PLT and ANC 1mo
- Hgb, PLT and ANC 3mo
- Hgb, PLT and ANC 6 mo
- Date 1 mo

- Date 3mo
- Date 6mo
- IL-6 day 0
- IL-6 Max
- Day IL6- Max observed
- Received Transfusion Since Noted Cytopenia Post-Infusion
    - Platelets
    - RBC
- GCSF Post Infusion
- Infectious complications post infusion within 1 year
- List Prior Lines of Systemic Therapy
- Prior Radiation
- Location of Radiation
- Bone Marrow Biopsy post infusion
    - Date of bone marrow biopsy
    - MDS
    - AML
    - Comments on Cellularity
    - Dysplasia
    - Comments on cell lines
    - Cytogenetics

APPENDIX II: Evaluation and Grading Methods

Table 13. ECOG Performance Status

Grade	ECOG Performance Status
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physical strenuous activity by ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any selfcare; totally confined to bed or chair
5	Dead

Criteria used to determine effects of disease and treatment toxicities on a patient's daily life. Adapted from Oken et al., 1982.

Table 14. Risk Factors Involved in IPI

IPI Risk Factors
Age > 60
Ann Arbor stage III or IV
> 1 extranodal site
Serum lactate dehydrogenase (LDH) level above normal
ECOG performance status ≥2

Abbreviations: ECOG: Eastern Cooperative Oncology Group; IPI: International Prognostic Index
Five risk factors used to predict patient's level of risk from disease. The more risk factors equate to a higher risk patient. Adapted from M.A. Shipp et al., 1993.

Table 15. The International Prognostic Index

Risk Group	IPI Score	Percentage of Patients	5-year OS	CR Rate
Low	0-1	35%	73%	87%
Low-Intermediate	2	27%	51%	67%
High-Intermediate	3	22%	43%	55%
High	4-5	16%	26%	44%

The IPI is used to predict treatment outcome and risk of reoccurrence of disease. Adapted from M.A. Shipp et al., 1993.

Table 16. The Deauville Five Point Scale

Score	Definition
1	No uptake
2	Uptake ≤ mediastinum
3	Uptake > mediastinum but ≤ liver
4	Moderately increased uptake compared to the liver
5	Markedly increased uptake compared to the liver and/or new lesions
X	New areas of uptake unlikely to be related to lymphoma

The Deauville five point scale (D5PS) is used to standardize measuring of fluorodeoxyglucose (FDG) uptake in a patient. The FDG uptake is measured from the site with the most intense uptake and is compared to the values of FDG uptake of a patient's own mediastinum and liver. Adapted from Barrington and Kluge, 2017.

Table 17. The Lugano Staging System

Stage	Involvement	Extranodal (E) Status
Limited		
I	One node or a group of adjacent nodes	Single extranodal lesions without nodal involvement
II	Two or more nodal groups on the same side of the diaphragm	Stage I or II by nodal extent with limited contiguous extranodal involvement
II bulky*	II as above with "bulky" disease	Not applicable
Advanced		
III	Nodes on both sides of the diaphragm; nodes above the diaphragm with spleen involvement	Not applicable
IV	Additional noncontiguous extralymphatic involvement	Not applicable

The Lugano Staging System is used to help physicians standardize the extent of a patient's disease and can help guide in determine appropriate treatment. Adapted from Chenson et al., 2014.
*Whether stage II bulky disease is treated as limited or advanced disease may be determined by histology and a number of prognostic factors

Table 18. Tumor Response Classifications of the Lugano Criteria

Classification	PET/CT-Based Criteria	CT-Based Criteria
Complete response (CR)	1, 2, or 3 points on D5PS; No new lesions; No bone marrow involvement	Regression of nodal masses to greater than 1.5 cm along the longest transverse diameter; Regression of enlarged organs to normal size; No new lesions; No bone marrow involvement
Partial response (PR)	4 or 5 points on D5PS; Reduced uptake compared to baseline; No new lesions; Residual bone marrow uptake that is reduced from baseline	Greater than 50% reduction from baseline in the sum of the product of the perpendicular diameters of up to 6 nodes; Greater than 50% reduction from baseline in the size of an enlarged spleen; No new lesions
Stable disease (SD)	4 or 5 points on D5PS; Unchanged uptake compared to baseline; No new lesions; Unchanged bone marrow involvement	Less than 50% reduction from baseline in the sum of the product of the perpendicular diameters of up to 6 nodes; No new lesions
Progressive disease (PD)	4 or 5 on D5PS; Increased uptake compared to baseline; New or recurrent involvement in nodes and bone marrow demonstrated by 18F-FDG avidity	Greater than 50% increase in product of perpendicular diameters of a node; Increase in nodal diameter (by 0.5 cm if node is ≤ 2 cm, 1.0 cm if > 2 cm); New or recurrent splenomegaly; New or recurrent involvement of nodes and bone marrow

Abbreviations: D5PS: Deauville Five Point Scale; FDG: fluorodeoxyglucose
The Lugano tumor response classifications identify a patient's response to treatment. Taken from Moghbel et al., 2016.

Table 19. Lee CRS Revised Grading System

Grade	Toxicity
Grade 1	Symptoms are not life threatening and require symptomatic treatment only, eg, fever, nausea, fatigue, headache, myalgias, malaise
Grade 2	Symptoms require and respond to moderate intervention Oxygen requirement <40% or Hypotension responsive to fluids or low dose[2] of one vasopressor or Grade 2 organ toxicity
Grade 3	Symptoms require and respond to aggressive intervention Oxygen requirement ≥40% or Hypotension requiring high dose* or multiple vasopressors or Grade 3 organ toxicity or grade 4 transaminitis
Grade 4	Life-threatening symptoms Requirement for ventilator support or Grade 4 organ toxicity (excluding transaminitis)
Grade 5	Death

Grades 2-4 refer to CTCAE v4.0 grading.
*High-dose vasopressor doses shown in Table 3.

The Lee grading system is used to standardize the intensity of CRS and to help in distinguishing progression or improvement of CRS. Taken from Lee et al., 2014.[34]

Table 20. CTCAE ICANS Grading Criteria

CTCAE Term	Grade 1	Grade 2	Grade 3	Grade 4
Encephalopathy	Mild Symptoms	Moderate symptoms; limiting instrumental ADL	Severe symptoms; limiting self-care ADL	Life-threatening consequences; urgent intervention indicated
Definition: A disorder characterized by a pathologic process involving the brain.				
Seizure	Brief partial seizure and no loss of consciousness	Brief generalized seizure	New-onset seizures (partial or generalized); multiple seizures despite medical intervention	Life-threatening consequences
Definition: A disorder characterized by a sudden, involuntary skeletal muscular contractions of cerebral or brain stem origin.				
Dysphasia	Awareness of receptive or expressive characteristics; not impairing ability to communicate	Moderate receptive or expressive characteristics; impairing ability to communicate spontaneously	Severe receptive or expressive characteristics; impairing ability to read, write, communicate intelligibly	N/A
Definition: A disorder characterized by impairment of verbal communication skills, often resulting from brain damage.				
Tremor	Mild symptoms	Moderate symptoms; limiting instrumental ADL	Sever symptoms; limiting self-care ADL	N/A
Definition: A disorder characterized by the uncontrolled shaking movement of the whole body or individual parts.				
Headache	Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self-care ADL	N/A
Definition: A disorder characterized by a sensation of marked discomfort in various parts of the head, not confined to the area of distribution of any nerve.				
Confusion	Mild disorientation	Moderate disorientation; limiting instrumental ADL	Severe disorientation; limiting self-care ADL	Life-threatening consequences; urgent intervention indicated
Definition: A disorder characterized by a lack of clear and orderly thought and behavior.				

Table 20. CTCAE ICNS Grading Criteria (continued)

Depressed level of consciousness	Decreased level of alertness	Sedation; slow response to stimuli; limiting instrumental ADL	Difficult to arouse	Life-threatening consequences; coma; urgent intervention indicated
Definition: A disorder characterized by a decrease in ability to perceive and respond.				
Edema cerebral	N/A	N/A	New onset; worsening from baseline	Life-threatening consequences; urgent intervention indicated
Definition: A disorder characterized by swelling due to an excessive accumulation of fluid in the brain.				

ADL: Activities of daily living

The CTCAE grading criteria for ICANS. Adapted from Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0, 2017

LIST OF JOURNAL ABBREVIATIONS

Adv Cell Gene Ther ..... Advances in Cell and Gene Therapy

Am J Clin Oncol ..... American Journal of Clinical Oncology

Ann Diagn Pathol ..... Annals of Diagnostic Pathology

Ann Lymphoma ..... Annals of Lymphoma

Best Pract Res Clin Haematol ..... Best Practice and Research Clinical Haematology

Biol Blood Marrow Transplant ..... Biology of Blood and Marrow Transplantation

Blood Rev ..... Blood Reviews

Br J Haematol ..... British Journal of Haematology

Eur J Nucl Med Mol Imaging ..... European Journal of Nuclear Medicine and Molecular Imaging

J Clin Oncol ..... Journal of Clinical Oncology

J Nucl Med ..... Journal of Nuclear Medicine

Lancet Oncology ..... The Lancet. Oncology

Mol Ther – Methods Clin Dev ..... Molecular Therapy. Methods and Clinical Development

Nat Rev Clin Oncol ..... Nature Reviews. Clinical Oncology

N Engl J Med ..... New England Journal of Medicine

South Asian J Cancer ..... South Asian Journal of Cancer

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